

OF GREENISH *ENCYCLIA*: NATURAL VARIATION, TAXONOMY, CLEISTOGAMY, AND A COMMENT ON DNA BARCODING

FRANCO PUPULIN¹⁻⁵ & DIEGO BOGARÍN¹⁻⁴

¹ Lankester Botanical Garden, University of Costa Rica, P. O. Box 1031-7050 Cartago, Costa Rica

² Harvard University Herbaria, Cambridge, Massachusetts 02138 USA

³ Marie Selby Botanical Gardens, Sarasota, Florida 34236 USA

⁴ Andean Orchids Research Center “Ángel Andreetta”, University Alfredo Pérez Guerrero, Ecuador

⁵ Corresponding author: franco.pupulin@ucr.ac.cr

ABSTRACT. The species-level taxonomy of *Encyclia* has been disputed considerably because of the great morphological similarity among many of the taxa, particularly in the complex of species related to *E. chloroleuca* and *E. gravida*, characterized by small, greenish flowers. Current phylogenetic results are insufficient to assess the natural lineages of the greenish species of *Encyclia*, and species concepts in this group are discussed here independently from previous schemes of classification and current nomenclatural uses. In Mesoamerica, traditional taxonomic approaches shifted from broad views of species circumscriptions to the recognition of a large number of finely split taxa. However, the relative paucity of specimens available for study led both approaches to fail to appreciate the range of natural variation, with the consequence of nomenclatural inflation and misunderstanding of species diversity. On the basis of a better sample, we reduce the supposedly rare and variable *E. amanda* to synonymy of *E. chloroleuca* and discuss the case of cleistogamous individuals of *Encyclia* referred to *E. gravida*. On the basis of floral morphology, we suggest that the few documented records of *E. gravida* may simply represent self-pollinating forms belonging to different taxa.

RESUMEN. La necesidad de conceptos específicos delimitados rigurosamente ha sido enfatizada para contar con inventarios de biodiversidad más certeros. Sin embargo, mientras el número de nuevos organismos descritos crece con un ritmo que no tiene antecedentes, nuestros conocimientos de mucho de los antiguos y nuevos taxones está todavía basado en un número extremadamente reducido de muestras, con una apreciación en el mejor de los casos reducida de su variación natural. Aun cuando se consideren en su circunscripción más estrecha, cercana al concepto original propuesto por Hooker (es decir, plantas con pseudobulbos piriformes, una inflorescencia terminal que no nace de una espata, hojas coriáceas a carnosas, un labio membranáceo generalmente provisto de lóbulos laterales bien desarrollados que envuelven la columna, y un callo forcipato en el disco del labelo), las “verdaderas” especies de *Encyclia* siguen siendo un grupo taxonómico difícil. Esto es particularmente evidente para un amplio complejo de especies emparentadas con *E. chloroleuca*, caracterizadas por flores pequeñas y en su mayoría verdosas, que se distribuyen en todo el Neotrópico. Las interpretaciones taxonómicas tradicionales de este grupo en la región mesoamericana oscilan entre el reconocimiento de 13 taxones finamente partidos y la taxonomía más conservadora de Ames y sus colegas en Harvard, quienes adoptaron conceptos amplios y en el grupo reconocieron solamente cuatro especies (con cinco subespecies). Ambas interpretaciones, sin embargo, se basaron fundamentalmente en el estudio de material escaso, a menudo unas pocas colecciones por cada país, según un sistema que ha prevenido la apreciación de la variación natural y últimamente llevó los taxónomos hacia una inflación en la nomenclatura o, alternativamente, a un concepto generalmente equivocado de la diversidad de las especies. En Mesoamérica, hoy en día reconocemos nueve especies de *Encyclia* verdosas (una de ellas probablemente no descrita) algunas de las cuales se extienden a Suramérica. La taxonomía de los taxones Andinos de este grupo, sin embargo, necesita urgentemente de revisión. En los últimos años, la secuenciación del genoma de las plantas, sobretodo a través de los códigos de barras, se ha reconocido como una poderosa herramienta para evaluar la congruencia de los conceptos específicos y descubrir taxones crípticos difíciles de separar con métodos taxonómicos tradicionales. Sin embargo, los códigos de barras por si solos nunca son suficiente para describir una nueva especie, aunque a un cierto punto códigos muy claramente divergentes

pueden utilizarse (con otros conjuntos de datos) como base para decisiones nomenclatoriales. Para priorizar cuales “morfoespecies” y cuales individuos deben ser secuenciados en complejos de especies problemáticos, el análisis del rango de variación intra- e interespecífico constituye un primer paso necesario para ubicar los códigos de barras en el contexto de un acercamiento taxonómico complejo, que finalmente utilice las técnicas basadas en el ADN en conjunto con otras disciplinas, tales como la filogeografía, la morfología comparativa, la genética de poblaciones, la ecología, el desarrollo y la biología reproductiva, para delimitar de forma mejor las unidades de la diversidad de orquídeas.

KEY WORDS: Orchidaceae, Laeliinae, *Encyclia*, Central America, taxonomy, cleistogamy

The need for rigorously delimited species concepts has been emphasized for the accuracy of biodiversity inventories. However, while the number of newly described organisms increases at an unprecedented rate, our knowledge of many of the old and new taxa is still based on a minimal number of samples. This is particularly evident in the case of tropical orchids, an impressively diverse group of organisms, which are in many cases only fragmentarily known through a few records randomly collected over their geographic ranges. The proliferation of synonyms in species descriptions, which traditionally plagued orchid taxonomy, is largely due to the difficulty of appreciating the range of natural variation among and between populations on the basis of the limited available samples, particularly in the case of taxa with broad distributions. Before the advent of large floristic projects such as *Flora Neotropica* in the 1960s (to date, only the genera *Bulbophyllum*, *Epidendrum*, *Isochilus*, *Mormodes*, and *Vanilla* have been scheduled for inclusion) and *Flora Mesoamericana* in the last 15 years, floristics have been published mostly on a national or local scale, and widespread taxa have often received a different name in each of the countries where they naturally occur.

In the case of the Neotropical orchid genus *Encyclia* Hook., the interest of systematic botanists mostly focused in the past on a more convenient - or more natural - way to circumscribe the genus (e.g., Lindley 1842, 1853, Ames *et al.* 1936, Dressler, 1961, 1984, Dressler & Pollard 1971, 1974, Withner 1998, 2001, van den Berg *et al.* 2000, Higgins 1998, Higgins *et al.* 2003), but the task of describing the diversity of *Encyclia* was essentially achieved in a floristic context with little or no attention given to any scheme of relationships among species. Joseph D. Hooker established *Encyclia* in 1828 based on a species native to Rio de Janeiro, Brazil, and separated

it from “the splendid species of *Cattleya*,” at that time including only three species (Hooker 1828). Hooker’s *Encyclia viridiflora*, the type species for the genus, is indeed a peculiar and poorly known plant with many unique characteristics compared to its close relatives. These include non-resupinate flowers, a short column, the lateral lobes of the lip almost completely fused with the midlobe, and a nonforcipate basal callus (Hooker, 1828, Withner 1996; Fig. 1). By the midpoint of the 19th century, under the authority of John Lindley, the genus was merged under *Epidendrum* L., and the type species was assigned to



Figure 1. Original illustration of *Encyclia viridiflora*, from Curtis’s *Botanical Magazine* 1828, pl. 2851.

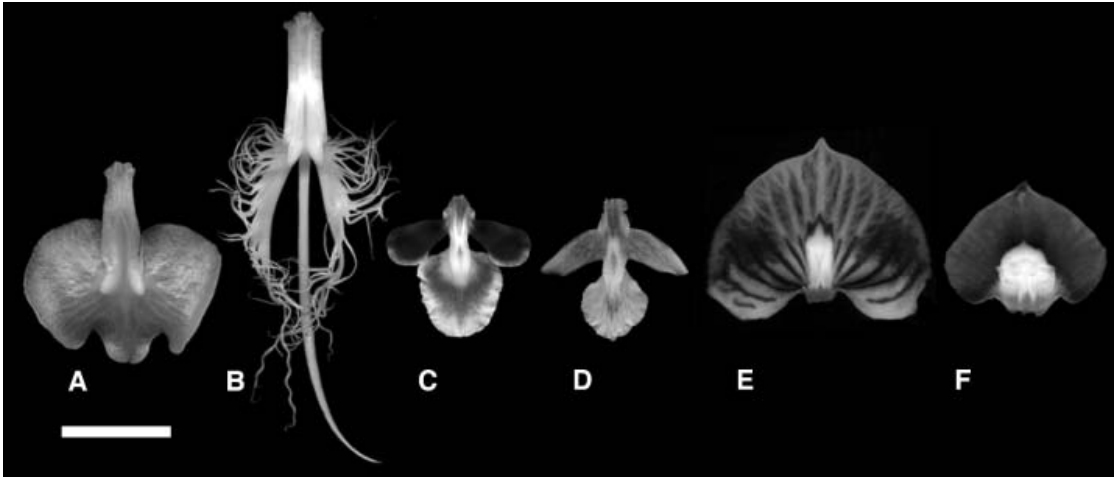


FIGURE 2. Labella of *Epidendrum*, *Encyclia* and *Prosthechea* species. A - *Epidendrum storkii*; B - *E. ciliare*; C - *Encycliamooreana*; D - *E. chloroleuca*; E - *Prosthechea cochleata*; F - *P. spondiada*. Scale bar = 1 cm.

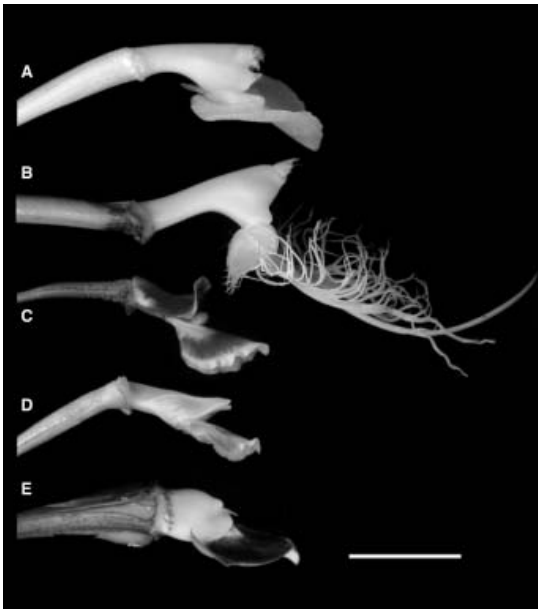


FIGURE 3. Lateral views of the gynostemium and labella of *Epidendrum*, *Encyclia* and *Prosthechea* species, showing various degrees of adnation between column and lip. A — *Epidendrum storkii*; B - *E. ciliare*; C - *Encycliamooreana*; D - *E. chloroleuca*; E - *Prosthechea spondiada*. Scale bar = 1 cm.

E. subgenus *Encyclium* Lindl. section *Hymenochyla* Lindl. (Lindley 1831, 1842, 1853). Despite attempts to revive *Encyclia* by Rudolf Schlechter (1914, 1918, 1922, 1923), it was not until 1961 when Robert L. Dressler offered conclusive evidence to recognize the

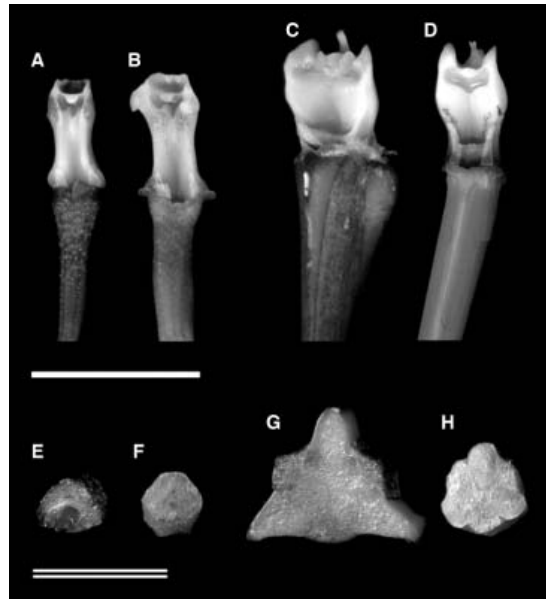


FIGURE 4. Columns and sections through the ovaries of *Epidendrum*, *Encyclia* and *Prosthechea* species. A-D, Columns. A - *Encyclia mooreana*; B - *E. chloroleuca*; C - *Prosthechea spondiada*; D - *P. cochleata*; E-H, Transverse sections through the ovaries. E - *Encyclia mooreana*; F - *E. chloroleuca*; G - *Prosthechea spondiada*; H - *P. cochleata*. Scale bar = 1 cm; double bar = 5 mm.

distinctness of the genus and the need to segregate it from *Epidendrum* (Dressler 1961; Fig. 2-3). However, even when the genus is considered in its narrower circumscription approximating the original concept by Hooker (Withner 1996), today widely

accepted notwithstanding the removal of the large bulk of species now assigned to *Prosthechea* Knowl. & Westc. (Higgins 1998; Fig. 4), the 'true' *Encyclia* species still form a difficult taxonomic group, and the identity of many of the species in the genus remains confused (see, for example, Dressler 2004 vs. Withner 1998, 2001).

The taxonomy of greenish *Encyclia*

Species-level taxonomy of *Encyclia* has been widely disputed, largely because of the great morphological similarity among many of the taxa — particularly in the large complex of species related to *E. chloroleuca* and *E. gravida* (Lindl.) Schltr., which are characterized by small and mostly greenish flowers — and the application of old names often based on poorly defined species concepts. We refer to these complexes here by the collective name of 'greenish encyclias'. 'Green' is not in any way a taxonomic category, and current phylogenetic results are insufficient to assess the natural lineages of the greenish species, so we feel free to discuss this group independently from any previous scheme of classification and current nomenclatural uses.

With several exceptions, which are easy to identify by unique sets of floral and vegetative features (such as the distinctive *E. adenocaula* (Llave & Lex.) Schltr., *E. cordigera* (Kunth) Dressler, or *E. phoenicea* (Lindl.) Neumann), species of *Encyclia* commonly share a common, uniform, and generalized morphological scheme. The plants are provided with ovoid pseudobulbs that bear two or three (rarely four) coriaceous leaves at the apex; inflorescences are frequently paniculate and variable in length even at different stages of the same individual, with the rachis and the pedicellate ovaries variously verruculose; flowers have three similar sepals, frequently clawed petals, a distinctly trilobed lip with the lateral lobes erect and the midlobe variously adorned with thickened veins, which in many taxa appear as longitudinal-radiating, sometimes prominent keels, and a basal, forcipate callus; the column is normally straight and provided with apical wings, which commonly embrace the isthmus that separate the lateral lobes from the midlobe. The perianth parts are frequently greenish or pale tan, with the lip cream-white, often provided with rose-purple

stripes along the main veins. Populations of *Encyclia* corresponding to this scheme are broadly distributed in the Neotropics, from Florida, Mexico, and the West Indies, throughout Central America, to Argentina and Paraguay, occurring both in Andean and Amazonian South America (Table 1).

For plants located in Mesoamerica, which we have investigated more closely during the past few years, traditional taxonomic approaches to understanding *Encyclia* have shifted from broad views of species circumscriptions that considered many of the described species of *Epidendrum* and *Encyclia* from Mesoamerica as a single polymorphic alliance (Ames *et al.*, 1935, 1936) to the recognition of a large number of finely split taxa (Withner, 1996). According to the different taxonomic treatments, the group of greenish Central American *Encyclia* includes 4 to 13 species. The use of broad concepts to avoid the difficulty of finding clear ways to distinguish between closely allied species reached its apex during the first half of the last century with the work by Oakes Ames and his co-workers at Harvard University. In their studies on the genus *Epidendrum* (including *Encyclia*) of North and Central America (Ames *et al.*, 1936), they considered most of the species with medium-sized flowers and an orbicular midlobe of the lip as varieties of a single variable species, a broadly defined *Epidendrum oncidoides* Lindl. However, scrutiny of more material now available proved that this gross lumping of Central American species under a few of the oldest names is untenable (Dressler and Pollard, 1976; Withner, 1996; Dressler, 2004; Pupulin, 2005, 2006). At the same time, a finely split concept for this group is equally untenable. The relative paucity of specimens available for study, together with the adoption of unconfirmed geographical records and the pronounced polymorphism of many Central American species, induced some botanists to adopt an excessively fine approach, often relying on subtle differences in type specimens that do not hold up in comparison with broader series of records (Withner, 1998, 2001). In both approaches, the impossibility of fully appreciating the range of natural variation eventually drove taxonomists to nomenclatural inflation or, alternatively, to a general misunderstanding of species diversity.

TABLE 1. Described species of *Encyclia* with greenish flowers.

Species	Distribution
<i>Encyclia acuta</i> Schltr.	Brazil, Venezuela
<i>Encyclia acutifolia</i> (Sw.) Nir	Jamaica
<i>Encyclia alanjensis</i> (Ames) Carnevali & Romero	Panama
<i>Encyclia alboxanthina</i> Fowlie	Brazil
<i>Encyclia amanda</i> (Ames) Dressler	Panama
<i>Encyclia amicta</i> (Lind. & Rchb.f.) Schltr.	Bolivia, Brazil, Paraguay, Peru, Venezuela
<i>Encyclia angustiloba</i> Schltr.	Bolivia, Ecuador, Peru
<i>Encyclia argentinensis</i> (Spegazzini) Hoehne	Argentina, Brazil, Paraguay
<i>Encyclia aspera</i> (Lindl.) Schltr.	Colombia, Ecuador, Peru
<i>Encyclia asperirachis</i> Garay	Colombia
<i>Encyclia asperula</i> Dressler & Pollard	Mexico, Guatemala, Belize
<i>Encyclia bradfordii</i> (Griseb.) Carnevali & Ramírez	Tobago, Trinidad, Venezuela
<i>Encyclia brenesii</i> Schltr.	Costa Rica
<i>Encyclia ceratistes</i> (Lindl.) Schltr.	El Salvador to Colombia and Venezuela
<i>Encyclia chloroleuca</i> (Hook.) Neumann	Belize to Brazil and Peru
<i>Encyclia confusa</i> Menezes	Brazil
<i>Encyclia davidhuntii</i> Withner & de Fuente	Belize, Guatemala, Honduras, Costa Rica
<i>Encyclia diurna</i> (Jacq.) Schltr.	Colombia, Suriname, Venezuela
<i>Encyclia expansa</i> (Rchb.f.) Ortíz	Colombia
<i>Encyclia fehlingii</i> (Sauleda) Sauleda & Adams	Bahamas
<i>Encyclia flava</i> (Lindl.) Porto & Brade	Brazil, Venezuela
<i>Encyclia fucata</i> (Lindl.) Britt. & Millsp.	Bahamas, Cuba
<i>Encyclia glandulosa</i> (Kunth) Ortíz	Colombia, Venezuela
<i>Encyclia gonzalensis</i> Menezes	Brazil
<i>Encyclia goyazensis</i> Menezes ex Fowlie	Brazil
<i>Encyclia granitica</i> (Lindl.) Schltr. Venezuela,	Guyana, Suriname, Brazil
<i>Encyclia guentheriana</i> (Kränzl.) R. Vásquez	Bolivia
<i>Encyclia guyanensis</i> Carnevali & Romero	Venezuela, Guyana, Suriname
<i>Encyclia hunteriana</i> Schltr.	Panama
<i>Encyclia inaguensis</i> Nash ex Britt. & Millsp.	Bahamas, Turks and Caicos Islands
<i>Encyclia leucantha</i> Schltr.	Colombia, Venezuela
<i>Encyclia lineariloba</i> Withner	Mexico, Guatemala, Nicaragua
<i>Encyclia linearifolioides</i> (Kränzl.) Hoehne	Brazil
<i>Encyclia longifolia</i> (Barb.Rodr.) Schltr.	Brazil
<i>Encyclia maderoi</i> Schltr.	Colombia
<i>Encyclia maravalensis</i> Withner	Trinidad
<i>Encyclia monticola</i> (Fawc. & Rendle) Acuña	Cuba, Hispaniola, Jamaica, Trinidad
<i>Encyclia mooreana</i> (Rolfe) Schltr.	El Salvador to Panama
<i>Encyclia mapiriensis</i> Kränzl.	Brazil
<i>Encyclia naranjapatensis</i> Dodson	Ecuador
<i>Encyclia nematocaulon</i> (A.Rich.) Acuña	Mexico to Nicaragua, and Cuba
<i>Encyclia odoratissima</i> (Lindl.) Schltr.	Brazil
<i>Encyclia oncidioides</i> (Lindl.) Schltr.	Brazil
<i>Encyclia ossenbachiana</i> Pupulin	Costa Rica
<i>Encyclia pachyantha</i> (Lindl.) Hoehne	Venezuela, French Guyana, Guyana, Brazil
<i>Encyclia patens</i> Hooker	Brazil
<i>Encyclia pauciflora</i> (Barb.Rodr.)	Porto & Brade Brazil
<i>Encyclia pedra-azulensis</i> Menezes	Brazil
<i>Encyclia peraltensis</i> (Ames) Withner	Costa Rica
<i>Encyclia picta</i> (Lindl.) Hoehne	Guyana

TABLE 1. *Continues.*

Species	Distribution
<i>Encyclia piracanjubensis</i> Menezes	Brazil
<i>Encyclia porrecta</i> B.R.Adams & P.J.Cribb	Belize
<i>Encyclia powellii</i> Schltr.	Panama
<i>Encyclia purpusii</i> Schltr.	Mexico
<i>Encyclia recurvata</i> Schltr.	Venezuela to Brazil
<i>Encyclia saltensis</i> Hoehne	Brazil
<i>Encyclia sclerocladia</i> (Lindl. ex Rchb.f.) Hoehne	Peru
<i>Encyclia steinbachii</i> Schltr.	Bolivia
<i>Encyclia stellata</i> (Lindl.) Schltr.	Costa Rica to Colombia and Venezuela
<i>Encyclia tampensis</i> (Lindl.) Small	Florida, Bahamas
<i>Encyclia tarumana</i> Schltr.	Brazil
<i>Encyclia thrombodes</i> (Rchb.f.) Schltr.	Bolivia, Peru, Brazil
<i>Encyclia tonduziana</i> Schltr.	Costa Rica
<i>Encyclia trachypus</i> Schltr.	Ecuador
<i>Encyclia triangulifera</i> (Rchb.f.) Acuña	Cuba
<i>Encyclia tripartita</i> (Vell.) Hoehne	Brazil
<i>Encyclia tuerckheimii</i> Schltr.	Mexico to Nicaragua
<i>Encyclia viridiflava</i> Menezes	Brazil
<i>Encyclia wagneri</i> Schltr.	Venezuela
<i>Encyclia xerophytica</i> Pabst.	Brazil
<i>Encyclia xypheres</i> (Rchb.f.) Schltr.	Mexico
<i>Encyclia yauaperyensis</i> (Vell.) Hoehne	Bolivia, Brazil
<i>Encyclia yucatanense</i> Schltr.	Mexico
<i>Epidendrum affine</i> Focke	Venezuela
<i>Epidendrum chloranthum</i> Lindl.	—
<i>Epidendrum halatum</i> Garay & Dunsterv.	Venezuela
<i>Epidendrum glutinosum</i> Scheidw.	Brazil
<i>Epidendrum ramonense</i> Rchb.f.	Costa Rica
<i>Epidendrum serronianum</i> Barb.Rodr.	Brazil
<i>Epidendrum spectabile</i> Focke	Venezuela

Size (of the sample) matters

One should emphasize that more common species, even in the group of the greenish *Encyclia*, are usually less taxonomically problematic. Taxonomy based on morphological analysis still relies on the interpretation of putative disjunctions along continuous variations of features, but this requires a representation of variation in character states as large as possible. In the Mesoamerican region, the ample records available for species such as *Encyclia ceratistes* (Fig. 5) or *E. mooreana* (Fig. 6) allow botanists not only to understand variation better, adopting sharper species circumscriptions and correctly interpreting heterotypic synonyms, but also detect those sister species that do not fit well any of the known specific patterns (Pupulin, 2006; Table 2).

A particularly critical situation is posed by taxa that span great geographic areas. As previously noted, floristic treatments have been mostly published on a national scale, and species comparison has been in many cases limited to the taxa recorded in immediately adjacent countries. Due to their overall diversity, the orchid floras of Central and South America have seldom been critically compared. It is a normal assumption that the patterns of geographic distribution in the Orchidaceae are generally defined by major continental masses, and the lack of generalist orchidologists has prevented in-depth monographic work spanning the whole Neotropics. When preparing a revision of *Encyclia* for the flora of Costa Rica (Pupulin and Bogarin, in preparation), we found one of these cases. Before the intensive field activity aimed at obtaining specimens for this study, *E. amanda* was known only

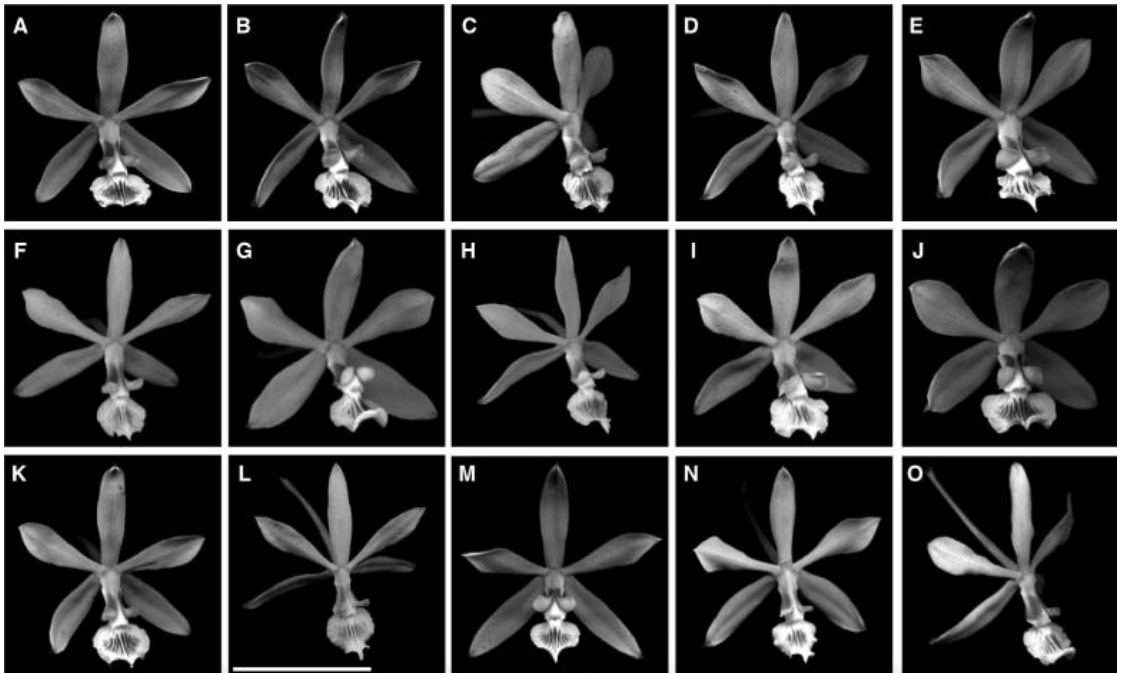


FIGURE 5. Floral variation in *Encyclia ceratistes*. A - Bogarín 3800; B - Bogarín 3799; C - Bogarín 3896; D - Bogarín 3797; E - Bogarín 3798; F - Pupulin 5641; G - JBL-s.n.; H - Pupulin 5200; I - Bogarín 5520; J - Bogarín 3806; K - Bogarín 3805; L - Pupulin 5303; M - Bogarín 3803; N - Pupulin 5641; O - Bogarín 3802. All the vouchers at JBL-Spirit. Scale bar = 2 cm.

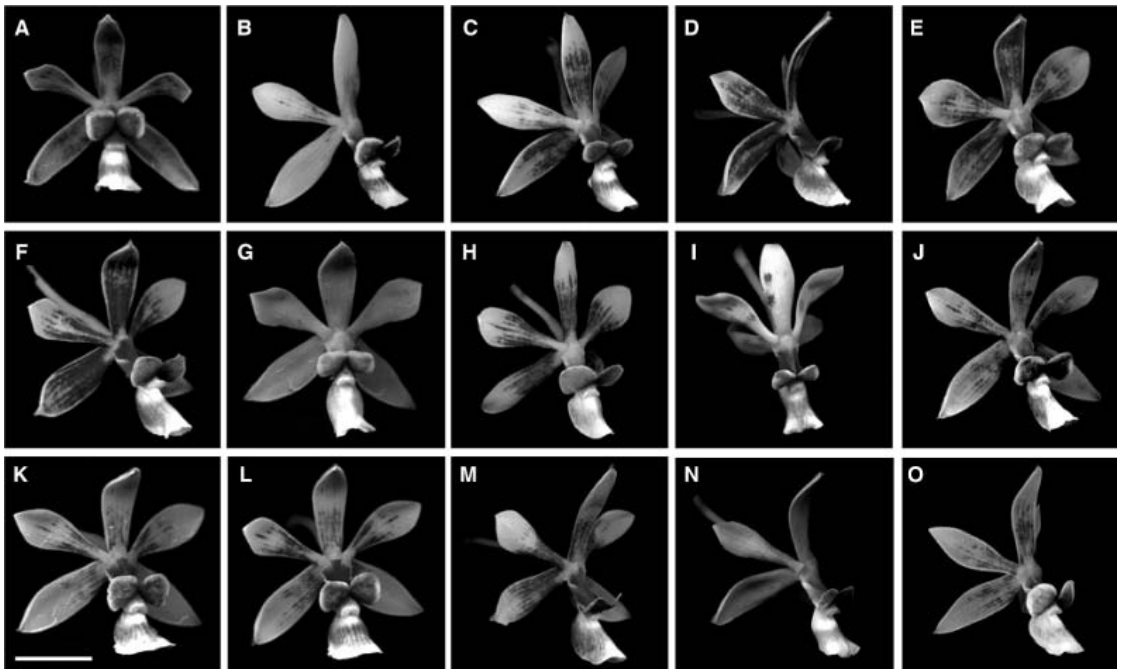


FIGURE 6. Floral variation in *Encyclia mooreana*. A - Bogarín 3810; B - JBL-06301; C - Bogarín 3721; D - JBL-08705; E - Bogarín 3787; F - Karremans 1356; G - JBL-08701; H - Gómez 3; I - Bogarín 3792; J - JBL-08704; K - Bogarín 3790; L - Bogarín 3791; M - JBL-08708; N - JBL-10044; O - JBL-08707. All the vouchers at JBL-Spirit. Scale bar = 1 cm.

TABLE 2. Comparison between taxonomic classifications of Costa Rican greenish *Encyclia*.

Williams 1946	Dressler & Pollard 1974	Dressler 1983	Withner 1998 & ss.	Dressler 2003	Pupulin & Bogarín (in prep.)
<i>Epidendrum oncioides</i> var. <i>gravidum</i>	<i>Encyclia gravida</i>	<i>Encyclia amanda</i>	<i>Encyclia amanda</i>		<i>Encyclia chloroleuca</i>
	<i>Encyclia alanjensis</i>		<i>Encyclia davidhunthii</i>		
	<i>Encyclia hunteriana</i>	<i>Encyclia gravida</i>	<i>Encyclia gravida</i>	<i>Encyclia stellata</i>	<i>Encyclia gravida</i> <i>Encyclia stellata</i>
			<i>Encyclia peraltensis</i>	<i>Encyclia peraltensis</i>	<i>Encyclia peraltensis</i>
<i>Epidendrum oncioides</i> var. <i>ramonense</i>	<i>Encyclia ceratistes</i>	<i>Encyclia ceratistes</i>	<i>Encyclia ceratistes</i>	<i>Encyclia ceratistes</i>	<i>Encyclia ceratistes</i>
<i>Epidendrum oncioides</i> var. <i>mooreanum</i>		<i>Encyclia mooreana</i>	<i>Encyclia mooreana</i>	<i>Encyclia mooreana</i>	<i>Encyclia mooreana</i>
	<i>Encyclia tuerckheimii</i>	<i>Encyclia tuerckheimii</i>	<i>Encyclia tuerckheimii</i>		
					<i>Encyclia ossenbachiana</i>

from a limited number of specimens. Withner (1998) mentioned no more than eight collections in the world's herbaria and included Costa Rica in the species distribution with no specimen citation (Withner, 2001). On the basis of the studied records, he characterized the species by the few-flowered (4 or 5), inflorescences that barely surpass the length of the leaves. Our collections revealed that this taxon is common in Costa Rica (Fig. 7) but extremely variable both in plant architecture and floral morphology. Well-developed inflorescences are commonly paniculate, many-flowered (12-30) and 60-70 cm long, but juvenile specimens may flower with short, simple, few-flowered racemes to only 15 cm long. Flowers vary greatly both within and among populations, and cultivated specimens show that floral variation may be notorious even on the same specimen at different flowering times (Fig. 8). When this range of variation is taken in account, the common Costa Rican taxon is not distinguishable from South American specimens of *Encyclia chloroleuca* (which has nomenclatural priority), and the study of the type specimen of *Epidendrum chloroleucum* at Kew confirmed our suspicions (Fig. 9). However, on the basis of the materials at our disposition, it is quite possible that the name *E. amanda* should be maintained for a distinct species from Panama and, perhaps, northern Colombia. The taxonomy of the Andean and Brazilian taxa in the group is still in urgent need of revision, and we cannot judge at this point how many specific epithets from these regions should be reduced to synonymy with *E. chloroleuca*.

Cleistogamous *Encyclia*

Even more intriguing and taxonomically difficult are those cases in which materials are hard to find due to the intrinsic nature of the relevant species. Now that we are concluding our revision of Costa Rican *Encyclia*, we will accept the concept of *E. gravida* with some reservation concerning the application of the name and the real identity of this taxon. Lindley (1849) originally described *Epidendrum gravidum* on the basis of a Mexican collection by Hartweg (*Hartweg s.n.*, the type!). The holotype at Kew (Fig. 10) consists only of a single inflorescence originally bearing four fruits (one of which was removed in 1923 and sent to Oakes Ames for study); the remaining sepals measure 10-11 mm in length and ca. 3 mm in width (Lindley, 1849; see also Carnevali *et al.*, 1994). Lindley (1849) considered it a "mere botanical curiosity," and in the protologue he gave no information about the vegetative characters of the new taxon. Florally, it was characterized by the linear lateral lobes of the lip, the ovate, acute midlobe provided with thickened veins, and the column with stigmatic, inflexed wings. After the original description, the name has been mostly adopted to identify populations of *E. stellata*, which shares with *E. gravida* a distinctly papillate ovary and the presence of prominent veins on the lip, but in a few cases the material referred to seems to match the original concept of Lindley.

Cleistogamy is a breeding system defined as the production of permanently closed, self-pollinated

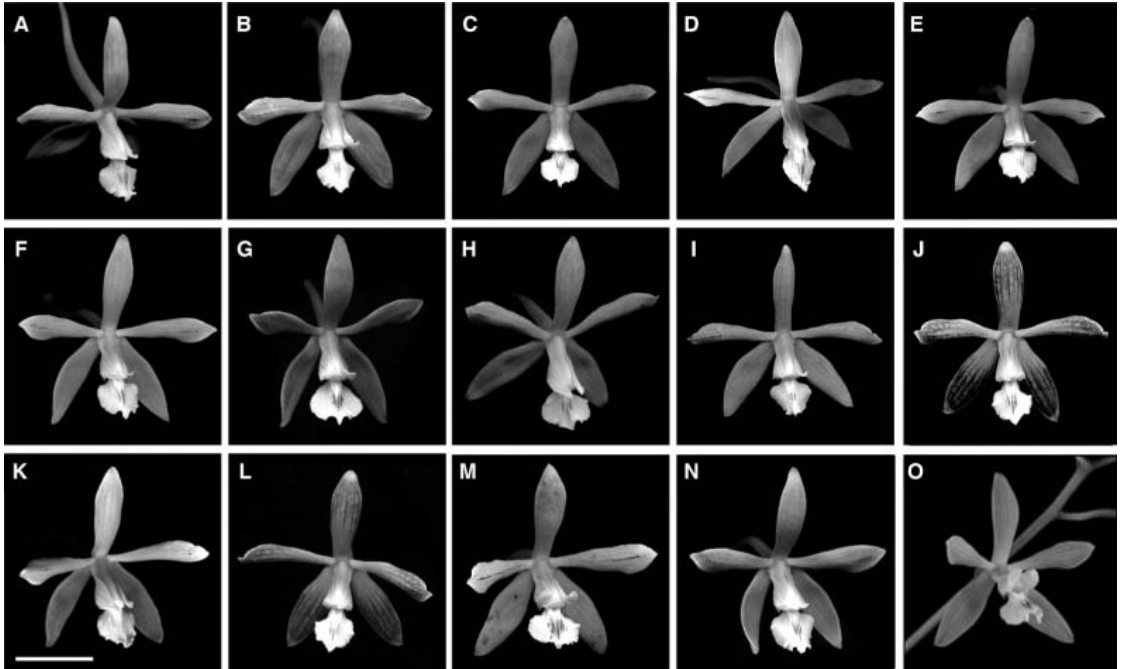


FIGURE 7. Floral variation in *Encyclia chloroleuca*. A - Bogarín 2537; B - Bogarín 2532; C - Bogarín 2537; D - Bogarín 3111; E - Bogarín 2544; F - Pupulin 3043; G - Ossenbach 365; H - Bogarín 2537; I - Bogarín 2544; J - Pupulin 3045; K - Pupulin 6536; L - Pupulin 3045; M - Pupulin 3045; N - Pupulin 3043; O - Pupulin 3044. All the vouchers at JBL-Spirit. Scale bar = 1 cm.

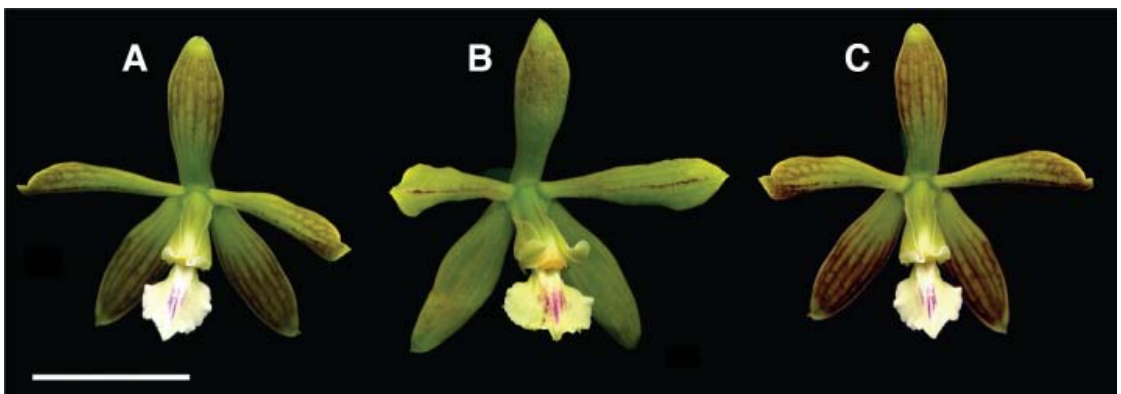


FIGURE 8. Floral variation in a single specimen of *Encyclia chloroleuca*. *Pupulin 3045*, flowering on 26 January 2003 (A), 11 June 2003 (B), and 3 March 2004 (C). Vouchers at JBL-Spirit. Scale bar = 1 cm.

flowers, and it is now recognized as an important system found in a variety of plant taxa (Culley and Klooster, 2007). Fertilization within cleistogamous flowers occurs without the intervention of pollinators, and in the Orchidaceae this is usually accomplished by direct transfer of pollen grains from anther to stigma. In cleistogamous species, selfpollinating flowers may

be the only type produced, but they may also appear together on the same plant along with typically insect-pollinated flowers. Complete cleistogamy, defined as the production of only cleistogamous flowers on an individual, has been especially reported in orchid species, but most indications are based on observations of only a few individuals.

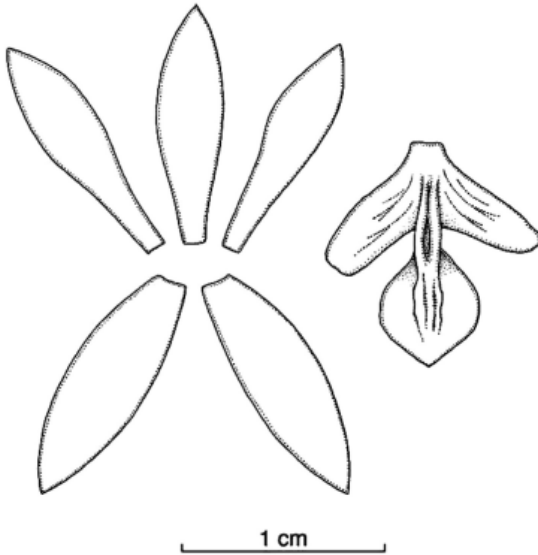


FIGURE 9. Tracing of a flower from the holotype of *Epidendrum chloroleucum*. Drawn with the aid of a camera lucida by D. Bogarín.

There are obvious difficulties in documenting “true” cleistogamous *Encyclia* individuals. Plants of other species related to “*E. grvida*” are frequently pollinated in the wild, and the pollination rate is high for several taxa. In Costa Rica, where large populations of *E. ceratistes* are found in pristine and altered habitats, it is not uncommon to observe inflorescences bearing literally tens of fruits. As these individuals are vegetatively indistinguishable from cleistogamous forms, the only way to tell the two entities apart is a careful observation of the perianth parts. In true cleistogamous specimens, the ovary begins to swell and elongate while the buds are in their early stage of development and the sepals are still turgid when the fruit approaches maturity, whereas in allogamous, post-anthesis fecund flowers the perianth remains in place but begins to dry out a few days after pollination and becomes papyraceous with age. We found that there is no way to distinguish between the fruit of a cleistogamous plant and the developing fruit of a recent pollinated flower, the perianth of which is still turgid, without opening the perianth and looking at the remnant of the rostellum, which is completely absent in cleistogamous forms. This may perhaps explain why the number of documented specimens of ‘gravid’ encyclias is scanty.

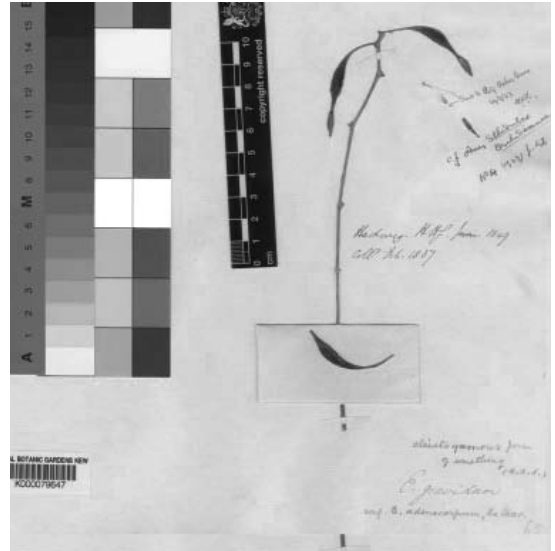


FIGURE 10. The holotype of *Epidendrum gravidum*. Reproduced with permission by the Board of Trustees, Royal Botanic Gardens, Kew.

Before World War II, Blanche Ames (Ames, 1923) illustrated the flower of another Mexican specimen collected by Purpus in Zacualpan, Veracruz, at that time kept in Schlechter’s herbarium in Berlin but later destroyed (Fig. 11, A), which may correspond morphologically to the species originally described by Lindley. A Puerto Rican cleistogamous specimen was documented by Ackerman (1995), who noted that the perianth parts of the opened flower are not exactly zygomorphic, probably because of some constraint during development (Fig. 11, B). It is likely that another collection recorded by Schweinfurth (1940) from Maricao in Puerto Rico (*Keveorkian 6177*, AMES) belongs to this concept, because, according to the collector, the “flowers [...] does not open.”

We have documented two cleistogamous specimens from plants growing in the living collections of Lankester Botanical Garden, University of Costa Rica. We had the plants growing under controlled conditions for a while, and cleistogamy in this case appears to be genetically controlled. Even though the two individuals share some of the morphological features expected for this taxon, they also differ in a number of characters, in particular the shape of the petals, the length of the labellar isthmus, the relative thickening of the veins on the midlobe, and the shape of the midlobe, which

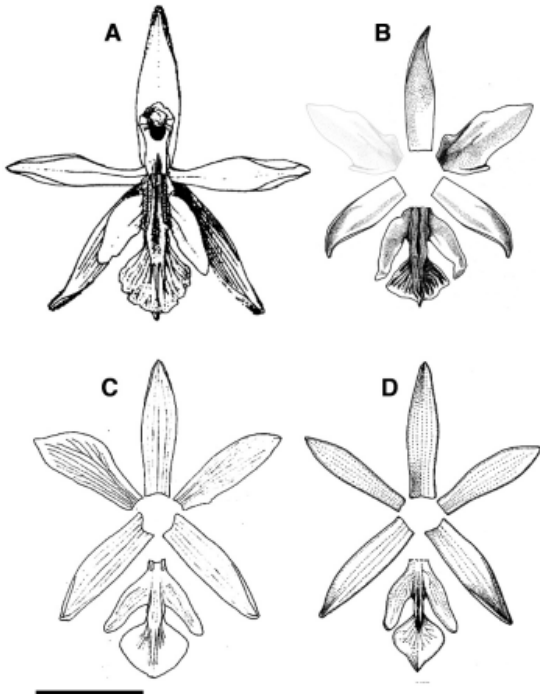


FIGURE 11. Cleistogamous *Encyclia*. A - Mexico, *Purpus s.n.*, (B, destroyed); B - Puerto Rico; C - Costa Rica, *Pupulin 6670* (JBL-Spirit); D - Costa Rica, *Pupulin 5377* (JBL-Spirit). A, drawn by Blanche Ames, from Ames, 1923; B, drawn by M. Enríquez, from Ackerman 1995; C-D, drawn by F. Pupulin. Scale bar = 1 cm.

is almost rhombic in one specimen and transversely ovate in the other (Fig. 11, C—D). More interesting is the fact that whereas one of the specimens lacks locality data, the other was collected in a region where only one other species of *Encyclia*, *E. ceratistes*, occurs naturally. During field work for the present treatment, we are making extensive collections of *Encyclia* specimens in this region, and to date we have observed only a single cleistogamous specimen. This may perhaps indicate that ‘gravid’ encyclias do not form populations, one of the essential requirements to be considered a good species, and are nothing more than occasional mutants.

When we compare the few documented specimens of cleistogamous *Encyclia*, it is evident that their similarity is superficial (Fig. 11, A-D), even when we could attribute these differences to some degree of deformity of the perianth parts, which do not spread out at the end of development. But which character (or set of characters) should properly define *E. grvida*?

Apart from the vegetative architecture, which is largely uniform in *Encyclia* species, and the features of the floral parts, which in turn vary considerably among individuals, the only shared character we observed in cleistogamous flowers is the absence of a definite rostellum, which would prevent the displacement of the pollen toward the stigmatic cavity. However, due to the paucity of available records of fecund plants of *Encyclia* to compare, this key feature alone is insufficient to assign cleistogamous individuals to a well-characterized species, and the possibility that the records simply represent self-pollinating forms belonging to different taxa cannot be discarded on the basis of floral morphology. The comparison of genetic sequences of individuals tentatively assigned to *E. grvida* with those of other sympatric species of *Encyclia* could represent an important step toward a better circumscription of the taxa in this group and the appreciation of the evolutionary significance of different pollination strategies.

A case for barcoding

In recent years, sequencing of plant genomes has been regarded as a powerful tool to assess the consistency of specific concepts and disclose cryptic taxa difficult to tell apart with traditional taxonomic methods, mainly through species DNA barcoding. However, even if at some stage clearly divergent barcodes can be used as the basis for nomenclatural decisions, barcodes by themselves are insufficient to describe new species. Species descriptions are scientific hypotheses that should be supported by a variety of other, including non-molecular, data sets. One of the necessary steps to place barcoding within the context of a rich taxonomic approach is the analysis of the range of intra- and interspecific variations in morphological characters, helping to prioritize which morphospecies and which individuals in problematic species complexes should be sequenced.

Application of the same species concept in taxonomically difficult groups like *Encyclia*, where hybridization may be frequent and occur over large areas, is still debatable (see Dressler and Pollard, 1976). DNA-based techniques may represent a unique opportunity to improve and simplify our hypotheses about species, but it is advisable they be used together with other disciplines such as phylogeography,

comparative morphology, population genetics, ecology, development, and pollination biology to delimit the units of orchid diversity.

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LITERATURE CITED

- Ackerman, J. D. 1995. An orchid flora of Puerto Rico and the Virgin Islands. *Mem. New York Bot. Gard.* 73: iii-203.
- Ames, O. 1923. Additions to the orchid flora of Central America with observations on noteworthy species. *Schedul. Orch.* 4: 1-62.
- Ames, O., Hubbard, F. T., and Schweinfurth, C. 1935. A fourth polymorphic alliance in *Epidendrum*. *Bot. Mus. Leaf.* (Harvard University) 3: 93-112.
- Ames, O., Hubbard, F. T., and Schweinfurth, C. 1936. *The genus Epidendrum in the United States and middle America*. Botanical Museum, Cambridge, UK.
- Carnevali, G., Ramírez, I. M., and Romero, G. A. 1994. Orchidaceae Dunstervilleorum VIII: species and combinations from Venezuelan Guayana. *Lindleyana* 9: 59-70.
- Culley, T. M. and Klooster, M. R. 2007. The cleistogamous breeding system: a review of its frequency, evolution, and ecology in angiosperms. *Bot. Rev.* 73: 1-30.
- Dressler, R. L. 1961. A reconsideration of *Encyclia* (Orchidaceae). *Brittonia* 13: 253-266.
- Dressler, R. L. 1984. La delimitación de géneros en el complejo *Epidendrum*. *Orquidea* (Mexico City) 9: 277-298.
- Dressler, R. L. 2004. A key to the Central American species of *Encyclia*. *Orch. Digest* 68: 88-91.
- Dressler, R. L. and Pollard, G. E. 1971. Nomenclatural notes on the Orchidaceae. IV. *Phytologia* 21: 433-439.
- Dressler, R. L. and Pollard, G. E. 1974. *The genus Encyclia in Mexico*. Asociación Mexicana de Orquideología, Mexico City, Mexico.
- Higgins, W. E. 1997 [1998]. A reconsideration of the genus *Prosthechea* (Orchidaceae). *Phytologia* 82: 370-383.
- Higgins, W. E., van den Berg, C., and Whitten, W. M. 2003. A combined molecular phylogeny of *Encyclia* (Orchidaceae) and relationships within Laeliinae. *Selbyana* 24: 165-179.
- Hooker, J. D. 1828. *Encyclia viridiflora*. *Curtis's Bot. Mag.* 55: pl. 2831.
- Lindley, J. 1831. *Encyclia*. Genera and species of orchidaceous plants, 111. Ridgways, London, UK.
- Lindley, J. 1842. *Epidendrum*. *Edwards's Bot. Reg.* 28: 27-36.
- Lindley, J. 1849. New plants, etc. from the Society's garden: 13. *Epidendrum gravidum*. *J. Hort. Soc. London* 4: 11.
- Lindley, J. 1853. Folia Orchidacea. *Epidendrum*. J. Matthews, London, UK.
- Pupulin, F. 2005. *Encyclia* Hook. In *Vanishing beauty — native Costa Rican orchids 1* (ed. F. Pupuli), pp. 256-267. Editorial de la Universidad de Costa Rica, San José, Costa Rica.
- Pupulin, F. 2006. *Encylia ossenbachiana* (Orchidaceae: Laeliinae), a new species from Costa Rica. *Selbyana* 27: 4-7.
- Schlechter, R. 1914. *Encyclia* Hook. In Schlechter, R., *Die Orchideen*, pp. 207-212. Paul Parey, Berlin, Germany.
- Schlechter, R. 1918. Kritische Aufzählung der bisher aus Zentral-Amerika bekanntgewordenen Orchideen; E. Aufzählung der Gattungen und Arten, part II (*Epidendrum-Stanhopea*). *Beih. Bot. Centralbl.* 36: 421-520.
- Schlechter, R. 1922. Beiträge zur Orchideenkunde von Zentralamerika. I. Orchidaceae Powellianae Panamenses. *Repert. Sp. Nov. Regni Veg. Beih.* 17.
- Schlechter, R. 1923. Beiträge zur Orchideenkunde von Zentralamerika. II. Additamenta ad Orchideologiam Costaricensis. *Repert. Sp. Nov. Regni Veg. Beih.* 19.
- Schweinfurth, C. 1940. A notable extension of range of *Epidendrum oncidioides* var. *gravidum*. *Bot. Mus. Leaf.* (Harvard University) 8: 187.
- Van den Berg, C., Higgins, W. E., Dressler, R. L., Whitten, W. M., Soto Arenas, M. A., Culham, A., and Chase, M. W. 2000. A phylogenetic analysis of Laeliinae (Orchidaceae) based on sequence data from internal transcribed spacers (ITS) of nuclear ribosomal DNA. *Lindleyana* 15: 96-114.
- Withner, C. 1996. *The cattleyas and their relatives. Vol. 4. The Bahamian and Caribbean species*. Timber Press, Portland, Oregon, USA.
- Withner, C. 1998. *The cattleyas and their relatives. Vol. 5. Brassavola, Encyclia, and other genera of Mexico and Central America*. Timber Press, Portland, Oregon, USA.
- Withner, C. 2001. *Encyclia* in Zentralamerika, zwei neue und sechs wenig bekannte Arten. *Orchidee* (Hamburg) 52: 444-450.